LISTING OF THE CLAIMS

The following listing of the claims replaces all prior versions and listings of claims for this application. Within this listing of the claims, claims 1, and 3-35 are pending, claims 1, 27, and 28 are amended, and claims 36 and 37 are new.

1. **(Currently amended)** A method for *in situ* detection of a nucleic acid analyte within a sample of biological material using bDNA hybridization comprising the steps of:

(a) preparing the sample of biological material by:

(i) immobilizing the biological material on a substrate,

(ii) permeabilizing the substrate-bound biological material by contacting the substrate-bound biological material to a solution containing Proteinase K at a concentration of about 0.5 μg/ml to about 50 μg/ml, ~~and~~

(iii) ~~optionally~~ digesting any RNA in the sample with RNase; and

(iv) heating the permeabilized biological material to a temperature and for a time period effective to denature any double-stranded DNA;

(b) contacting the biological material with a target oligonucleotide probe under hybridizing conditions, wherein at least a portion of the target probe is complementary to at least a portion of the nucleic acid analyte, so that an analyte-target probe complex is formed when the nucleic acid analyte is present in the sample;

(c) washing the biological material with a washing fluid comprising a detergent, at a temperature in the range of approximately 21 to 60°C; and

(d) detecting any analyte-target probe complex on the substrate by:

(i) contacting the washed substrate and analyte-target probe complex with a preamplifier oligonucleotide probe under hybridizing conditions, wherein a first portion of the preamplifier probe is complementary to a portion of the target probe other than the portion of the target probe that is complementary to the nucleic acid analyte, thereby forming an analyte-target probe-preamplifier probe complex when the nucleic acid analyte is present in the sample,

(ii) contacting the product of step (d)(i) with an amplifier oligonucleotide probe under hybridizing conditions, wherein a first portion of the amplifier probe is complementary to a second portion of the preamplifier probe, thereby forming an analyte-target probe-preamplifier probe-amplifier probe complex when the nucleic acid analyte is present in the sample,

(iii) contacting the product of step (d)(ii) with a label probe comprised of an alkaline phosphatase conjugated (AP-conjugated) oligonucleotide probe under hybridizing conditions, wherein a portion of the label probe binds to a second portion of the amplifier probe, thereby forming an analyte-target probe-preamplifier probe-amplifier probe-label probe complex when the nucleic acid analyte is present in the sample,

(iv) labeling the analyte-target probe-preamplifier probe-amplifier probe-label probe complex with a detectable label comprised of Fast Red, and

(v) detecting the presence of the label on the substrate,

wherein the nucleic acid analyte is selected from the group consisting of DNA, endogenous genes, and segments thereof.

2. **(Canceled)**

3. **(Previously presented)** The method of claim 1, wherein the nucleic acid analyte is selected from the group consisting of HIV DNA, CMV DNA, HPV DNA, LAP, and IL-2.

4. **(Original)** The method of claim 1, wherein the substrate comprises glass or a resilient plastic.

5. **(Original)** The method of claim 1, wherein the concentration of Proteinase K is from about 5 μg/ml to about 20 μg/ml.

6. **(Original)** The method of claim 1, wherein approximately 0.1 pmoles to 10 pmoles of the target probe is used.

7. **(Original)** The method of claim 1, wherein the detergent in the washing fluid is a hydrophilic surfactant.

8. **(Original)** The method of claim 7, wherein the hydrophilic surfactant is non-ionic.

9. **(Original)** The method of claim 1, wherein the washing fluid is a buffer solution.

10. **(Previously presented)** The method of claim 9, wherein the buffer solution comprises the salts of alkali metals.

11. **(Original)** The method of claim 1, wherein step (c) is repeated at least once.

12. **(Original)** The method of claim 11, wherein step (c) is repeated at least twice.

13. **(Canceled)**.

14. **(Previously presented)** The method of claim 1, wherein approximately 1 fmole to about 10 pmoles of the preamplifier oligonucleotide probe is used.

15. **(Previously presented)** The method of claim 1, wherein approximately 1 fmole to about 10 pmoles of the amplifier oligonucleotide probe is used.

16-17. **(Canceled)**

18. **(Original)** The method of claim 1, having a sensitivity sufficient to detect from 1 to about 10 copies of the nucleic acid analyte.

19. **(Original)** The method of claim 18, having a sensitivity sufficient to detect from 1 to about 2 copies of the nucleic acid analyte.

20. **(Original)** The method of claim 1, wherein the biological sample comprises a cell.

21. **(Original)** The method of claim 20, wherein the cell is isolated from the group consisting of plasma, serum, spinal fluid, semen, lymph fluid, the external sections of the skin, secretions of the respiratory tract, secretions of the intestinal tract, secretions of the genitourinary tract, tears, saliva, milk, blood cells, tumors, organs and in vitro cell culture constituents.

22. **(Original)** The method of claim 20, wherein the cell is selected from the group consisting of adrenal, bladder, bone marrow, brain, breast, cardiac, colon, esophageal, intestinal, kidney, liver, pulmonary, lymph node, nerve, ovarian, pancreatic, prostatic, skeletal muscle, smooth muscle, spleen, stomach, testicular, tonsil, tracheal and uterine cells.

23. **(Original)** The method of claim 20, wherein step (a)(i) is carried out using a centrifuge.

24. **(Original)** The method of claim 1, wherein the biological sample comprises a tissue.

25. **(Original)** The method of claim 24, wherein the tissue is selected from the group consisting of adrenal, bladder, bone marrow, brain, breast, cardiac, colon, esophageal, intestinal, kidney, liver, pulmonary, lymph node, nerve, ovarian, pancreatic, prostatic, skeletal muscle, smooth muscle, spleen, stomach, testicular, tonsil, tracheal and uterine tissues.

26. **(Original)** The method of claim 24, wherein step (a)(i) is carried out using sections of the tissue.

27. **(Currently amended)** A method for identifying the position of a nucleic acid analyte within a cell using bDNA hybridization comprising the steps of:

(a) preparing a sample of biological material by:

(i) immobilizing the biological material on a substrate,

(ii) permeabilizing the substrate-bound biological material by contacting the substrate-bound biological material to a solution containing Proteinase K at a concentration of about 0.5 μg/ml to about 50 μg/ml, and

(iii) optionally heating the permeabilized biological material to a temperature and for a time period effective to denature any double-stranded DNA or to remove mRNA secondary structure;

(b) contacting the biological material with a target oligonucleotide probe under hybridizing conditions, wherein at least a portion of the target probe is complementary to at least a portion of the nucleic acid analyte, so that an analyte-target probe complex is formed when the nucleic acid analyte is present in the sample;

(c) washing the biological material with a washing fluid comprising a detergent, at a temperature in the range of approximately 21 to 60°C;

(d) detecting any analyte-target probe complex on the substrate by:

(i) contacting the washed substrate and analyte-target probe complex with a preamplifier oligonucleotide probe under hybridizing conditions, wherein a first portion of the preamplifier probe is complementary to a portion of the target probe other than the portion of the target probe that is complementary to the nucleic acid analyte, thereby

forming an analyte-target probe-preamplifier probe complex when the nucleic acid analyte is present in the sample,

(ii) contacting the product of step (d)(i) with an amplifier oligonucleotide probe under hybridizing conditions, wherein a first portion of the amplifier probe is complementary to a second portion of the preamplifier probe, thereby forming an analyte-target probe-preamplifier probe-amplifier probe complex when the nucleic acid analyte is present in the sample,

(iii) contacting the product of step (d)(ii) with a label probe comprised of an alkaline phosphatase conjugated (AP-conjugated) oligonucleotide probe under hybridizing conditions, wherein a portion of the label probe binds to a second portion of the amplifier probe, thereby forming an analyte-target probe-preamplifier probe-amplifier probe-label probe complex when the nucleic acid analyte is present in the sample,

(iv) labeling the analyte-target probe-preamplifier probe-amplifier probe-label probe complex with a detectable label comprised of Fast Red, and

(v) detecting the presence of the label on the substrate; and

(e) identifying the position of the analyte-target probe complex within the cell, wherein the position of the analyte-target probe complex as determined by the presence of the label is indicative of the position of the nucleic acid analyte within the cell.

28. **(Currently amended)** A method for detecting a nucleic acid analyte within a sample of biological material comprising performing bDNA hybridization to detect the nucleic acid analyte *in situ*, wherein the method has a sensitivity sufficient to detect from about 1 to about 10 copies of the nucleic acid analyte in the biological material, the bDNA hybridization comprised of the steps of:

(a) preparing the sample of biological material by:

(i) immobilizing the biological material on a substrate,

(ii) permeabilizing the substrate-bound biological material by contacting the substrate-bound biological material to a solution containing Proteinase K at a concentration of about 0.5 μg/ml to about 50 μg/ml, and

(iii) optionally heating the permeabilized biological material to a temperature and for a time period effective to denature any double-stranded DNA or to remove mRNA secondary structure;

(b) contacting the biological material with a target oligonucleotide probe under hybridizing conditions, wherein at least a portion of the target probe is complementary to at least a portion of

the nucleic acid analyte, so that an analyte-target probe complex is formed when the nucleic acid analyte is present in the sample;

(c) washing the biological material with a washing fluid comprising a detergent, at a temperature in the range of approximately 21 to 60°C;

(d) detecting any analyte-target probe complex on the substrate by:

(i) contacting the washed substrate and analyte-target probe complex with a preamplifier oligonucleotide probe under hybridizing conditions, wherein a first portion of the preamplifier probe is complementary to a portion of the target probe other than the portion of the target probe that is complementary to the nucleic acid analyte, thereby forming an analyte-target probe-preamplifier probe complex when the nucleic acid analyte is present in the sample,

(ii) contacting the product of step (d)(i) with an amplifier oligonucleotide probe under hybridizing conditions, wherein a first portion of the amplifier probe is complementary to a second portion of the preamplifier probe, thereby forming an analyte-target probe-preamplifier probe-amplifier probe complex when the nucleic acid analyte is present in the sample,

(iii) contacting the product of step (d)(ii) with a label probe comprised of an alkaline phosphatase conjugated (AP-conjugated) oligonucleotide probe under hybridizing conditions, wherein a portion of the label probe binds to a second portion of the amplifier probe, thereby forming an analyte-target probe-preamplifier probe-amplifier probe-label probe complex when the nucleic acid analyte is present in the sample,

(iv) labeling the analyte-target probe-preamplifier probe-amplifier probe-label probe complex with a detectable label comprised of Fast Red, and

(v) detecting the presence of the label on the substrate.

29. **(Original)** The method of claim 28, wherein the nucleic acid analyte is selected from the group consisting of HIV RNA, HIV DNA, HCV RNA, CMV RNA, CMV DNA, HPV RNA, HPV DNA, LAP, IL-2, endogenous genes and segments thereof.

30. **(Original)** The method of claim 28, wherein the sensitivity is sufficient to detect from 1 to about 2 copies of the nucleic acid analyte.

31. **(Original)** The method of claim 28, wherein the biological material comprises a cell.

32. **(Original)** The method of claim 31, wherein the cell is isolated from the group consisting of plasma, serum, spinal fluid, semen, lymph fluid, the external sections of the skin, secretions of the respiratory tract, secretions of the intestinal tract, secretions of the genitourinary tract, tears, saliva, milk, blood cells, tumors, organs and in vitro cell culture constituents.

33. **(Original)** The method of claim 31, wherein the cell is selected from the group consisting of adrenal, bladder, bone marrow, brain, breast, cardiac, colon, esophageal, intestinal, kidney, liver, pulmonary, lymph node, nerve, ovarian, pancreatic, prostatic, skeletal muscle, smooth muscle, spleen, stomach, testicular, tonsil, tracheal and uterine cells.

34. **(Original)** The method of claim 28, wherein the biological sample comprises a tissue.

35. **(Original)** The method of claim 34, wherein the tissue is selected from the group consisting of adrenal, bladder, bone marrow, brain, breast, cardiac, colon, esophageal, intestinal, kidney, liver, pulmonary, lymph node, nerve, ovarian, pancreatic, prostatic, skeletal muscle, smooth muscle, spleen, stomach, testicular, tonsil, tracheal and uterine tissues.

36. **(New)** The method of claim 27, wherein the nucleic acid analyte is located within a subcellular compartment of the cell.

37. **(New)** The method of claim 28, wherein the signal and the nucleic acid analyte are co-localized within the subcellular compartment.